INVESTIGATION OF THE

NUTRITIONAL PROPERTIES OF *Hydrogenomonas eutropha*

Fourth

Semi-Annual Report

to

National Aeronautics and Space Administration

on

NASA Grant NGR-05-003-089

Submitted By

Doris Howes Calloway
Principal Investigator
Professor of Nutrition

Sheldon Margen
Co-Principal Investigator
Professor of Human Nutrition

Department of Nutritional Sciences
University of California, Berkeley

1 June 1966 to 30 November 1966

Studies with *Hydrogenomonas eutropha,* a hydrogen-fixing bacterium, indicate that it may serve as a basis of a bioregenerative system for renewal of cabin atmosphere in extended space flights. Limited rat feeding studies have demonstrated high biological quality of the protein of one sample of bacteria, harvested from Battelle's continuous culture apparatus at log-phase growth.

Progress during this period:

Lipid Digestibility

Three lots of bacteria were received, from Dr. Leonard Bongers of the Research Institute for Advanced Studies. Sample RIAS 1 consists of about 600 grams of bacteria harvested from a nitrogen-deficient medium. Samples RIAS 2 and RIAS 3 are both labeled with carbon^{14}. Lot RIAS 2 is 15 grams of conventional cells and RIAS 3 is 15 grams of nitrogen-deprived cells. Unwashed sample RIAS 1 was analyzed and found to contain 8.03 g of nitrogen per 100 g of lyophilized cells and 23 g of lipid. Sample BMI 2, received previously from Battelle Memorial Institute, was also analyzed chemically; it contains 12% of nitrogen and 3% of water.

Lots RIAS 1 and BMI 2 were formulated into mouse diets in order to measure digestibility of the bacterial lipid and nitrogen digestibility and retention. Because measurement of lipid absorption was the primary purpose of this study, the sample RIAS 1 was used to provide 10% of bacterial lipid in the final diet.* This was achieved by mixing 45.3 g of dry bacteria with required

---

*In order to assure adequate absorption of fat-soluble vitamins and a supply of essential fatty acids all diets also contained 1% of corn oil.

amounts of vitamins and minerals and making the total to 100 g with addition of glucose and starch. The composition of the bacteria resulted in a final concentration of about 24% of "protein" (16% N_2 equivalent) in the diet. Sample BMI 2 was used at a lower level to provide the same amount of bacterial protein in the finished diet, and corn oil was added to equate lipid content. The control diet contained the same percentages of protein and lipid from casein and corn oil. (See Table 1.) A low protein, low fat diet was used to determine endogenous levels of fecal lipid and nitrogen and urinary nitrogen.

Each of the diets was fed to a group of 6 or 7 male albino mice, Swiss-Webster strain, for 10 days. Excreta and rejected diet were quantitatively recovered during a 5-day period of controlled food intake. Lipid was extracted from the feces and diet by an ethyl ether-petroleum ether-ethanol solvent system after mild hydrochloric acid hydrolysis and dried at 60° C in a vacuum oven. Nitrogen was determined by micro-Kjeldahl analysis. Percentage digestibility was computed as follows:

$$\frac{\text{Intake} - (\text{Fecal output of test group} - \text{Fecal metabolic output})}{\text{Intake}} \times 100$$

From the control diet, 99% of both lipid and protein were absorbed (Table 2). With the RIAS cells, digestibility was only 19% of total lipid; since 10% of the lipid was corn oil shown to be 99% absorbable, digestibility of bacterial lipid is indicated to be not more than 10%. The lipid contained in the BMI diet was 47% absorbed, or less than the amount of corn oil present. Nitrogen digestibility was 93% with the RIAS sample and 94% with the BMI, in good agreement with rat data for the comparable BMI sample. (See report dated 30 November 1965.)

Good protein digestibility in spite of poor fat digestion, indicating that the high-fat cells are not resistant to digestion, suggests that the lipid is

Table 1

Diets Fed to Mice for Digestibility Trials

Component	RIAS	BMI g/100 g diet	Control
Lipid-rich RIAS *H. eutropha*	43.5	----	----
Protein-rich BMI *H. eutropha*	----	30.0	----
Casein	----	----	30.0
Corn oil	----	6.0	10.0
Salts USP XIV plus $ZnCO_3$[a]	6.0	6.0	6.0
B-vitamins mixture[b]	1.0	1.0	1.0
Fat-soluble vitamins in corn oil[c]	1.0	1.0	1.0
Cornstarch	24.25	28.0	26.0
Glucose	24.25	28.0	26.0

[a]Two parts of zinc carbonate added to 598 parts of USP XIV salts mixture which is adequate for the rat with respect to minerals other than zinc.

[b]The sucrose mixture provided, in mg per 100 g diet: thiamine HCl, 0.25; riboflavin, 0.50; pyridoxine HCl, 0.25; niacin, 2.0; Ca pantothenate, 2.0; inositol, 10.0; para-aminobenzoic acid, 5.0; biotin, 0.01; folic acid, 0.10; vitamin B_{12}, 0.005; and choline Cl, 100.0.

[c]The corn oil mixture provided, per 100 g diet: 1776 I.U. vitamin A palmitate; 160 I.U. vitamin D_3; 1.82 mg α-tocopherol; and 0.20 mg vitamin K_3.

Table 2

Digestibility of *Hydrogenomonas eutropha* Lipid[a]

	RIAS Lipid-rich	BMI Protein-rich	Casein-Corn oil
Food eaten, g/mouse/5 days	27	30	34
Lipid digestibility, %	19	47	99
Nitrogen digestibility, %	93	94	99

[a]Each entry is mean of six mice per group.

apparently located within the cell and that the cell membrane is not made more resistant by the cultural conditions. There is no evidence that the cells have toxic properties in the brief test period but the diet was less well-accepted by the mice than that based on the BMI cells or casein.

Amino Acid Content

Amino acid composition of washed sample BMI 1 was determined (Beckman analyzer). Concentrations are, in g per 16 g of nitrogen: threonine, 4.52; lysine, 8.61; methionine, 2.69; isoleucine, 4.58; leucine, 8.52; phenylalanine, 3.96; tyrosine, 3.26; valine, 7.13; histidine, 2.48; arginine, 8.00; alanine, 8.80; aspartic acid, 9.57; glutamic acid, 11.17; glycine, 5.47; proline, 3.46; and serine, 3.47. Tryptophan was not present because acid was used for hydrolysis. This analysis accounts for 96% of the protein, if the protein contains 16% of nitrogen. This pattern of amino acids compares favorably with casein and supports the finding of equal biological value, reported earlier (November 1965). Proportionality patterns match those of the Battelle analysis, except for lysine which is much higher in our sample, but concentration is 1.5 times greater (Foster and Litchfield, Biotech. and Bioeng., 6: 441, 1964).

Mineral Content

There is great variability among samples with respect to mineral content, which may be due to selection of buffer and purity of ingredients of the media. Battelle samples (N = 4) vary per 100 g of dry solids, from 0.02 to 0.21 g calcium; 0.02 to 0.10 g magnesium, and 0.03 to 0.62 g potassium. One unwashed sample from Mississippi State University contains 0.2 g calcium, 0.2 g magnesium, and 0.91 g potassium; and the lipid-rich RIAS sample has

0.01 g calcium, 0.04 g magnesium, and 0.79 g potassium per 100 g dry solids.

Plans for Next Period:

Beta-hydroxybutyric acid will be purchased and fed to rats at various levels, in conjunction with a purified diet, to determine tolerance to the monomer. If substantial amounts are well-tolerated, methods may be sought to degrade the polymer formed by the bacterium.